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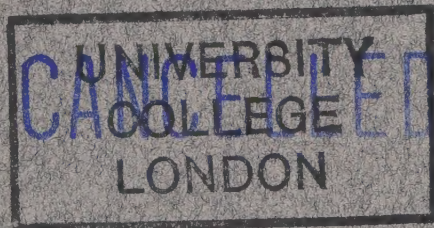
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NOTE UPON THE EFFECT OF STIMULATION OF THE
ACCELERATOR NERVE UPON THE CALCIUM,
POTASSIUM, AND NITROGEN METABO-
LISM OF THE ISOLATED HEART.

By W. H. HOWELL AND W. W. DUKE.

[FROM THE PHYSIOLOGICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY.]



MEDICAL SCIENCES

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[*From the Physiological Laboratory of the Johns Hopkins University.*]

IN a previous paper¹ the authors have shown, from experiments made upon the isolated heart, that there is an output of potassium from the heart substance during vagus inhibition. In the same paper we have suggested that during stimulation of the accelerator nerve there may be an output of calcium sufficient to account for the augmentation and, perhaps, for the acceleration of the beat.² During the past year we have attempted to test this last hypothesis by direct experiments made upon the perfused heart. It may be stated at once that the results were negative, but since the experiments were successful from a technical standpoint and were apparently conclusive in regard to the point in question, it seems desirable to place them upon record. Most of the experiments were made upon cats. The animals, as a rule, were heavily etherized and then bled by cutting the femorals. While bleeding, the heart was rapidly isolated by the method described in our previous paper. It was kept beating upon a supply of Locke's solution, fed to the coronary arteries at a temperature of 35° to 37° C. and under a relatively small pressure, 40 to 60 mm. Hg. The solution used had been previously thoroughly saturated with oxygen and was fed to the heart under oxygen pressure. In all of our later experiments we made use of the ingenious device described by Locke and Rosenheim³ for maintaining a continuous circulation of a small bulk of liquid in an atmosphere of oxygen.

¹ HOWELL and DUKE: This journal, 1908, xxi, p. 51.

² HOWELL and DUKE: Journal of physiology, 1906, xxxv, p. 131.

³ LOCKE and ROSENHEIM: Journal of physiology, 1907, xxxvi, p. 205

The method of procedure was as follows: After the heart had been isolated it was thoroughly washed out for half an hour or longer by warm Locke's solution supplied under oxygen pressure from a stock flask containing about 8 litres. The outflow from the heart during this process was allowed to waste, and the washing was continued until the outflow was perfectly clear and free from traces of blood. During this period the accelerator nerves were prepared for stimulation. In most cases we used the nerve on the left side. The stellate ganglion was exposed, and ligatures were placed round the conspicuous branch, usually quite distinct, which has been designated as the *nervus accelerans* by Boehm. After the heart had been thoroughly washed out from the stock flask the irrigation was transferred to a small special supply of the same solution, which was thereafter kept in continuous circulation through the heart. From 75 c.c. to 150 c.c. of Locke's solution were used for this purpose. The liquid as it emptied into the right auricle was received through a suitable cannula into a closed receiver immersed in warm water, and was then forced back into the supply flask by bubbles of oxygen from an oxygen tank, after the manner described by Locke. The stream of oxygen was first saturated with water vapor by passing through a long tube filled with moistened beads, and as the system was entirely closed there was no concentration from evaporation. By means of this device the heart can be kept beating with great vigor and regularity for many hours (five) upon a supply of 100 c.c. or less. In isolating the heart it was necessary to close off the pulmonary circuit, and this was effected by laying two stout ligatures round the roots of the lungs.

During the continuous circulation of the small supply of liquid the heart was repeatedly stimulated through the accelerator nerves, and at the end of the experiment samples were taken of the original solution, and of the solution which had been repeatedly perfused through the heart while under stimulation from the accelerator nerves. These samples were analyzed for their content in calcium and potassium. The samples were first slightly acidified with acetic acid and brought to a boil. The sample that had been circulated through the heart gave usually a slight opalescence or precipitate due to the presence, probably, of a small amount of coagulable protein. For analysis, 10 c.c. of the clear liquid was taken, evaporated to dryness, and ashed. The residue was treated with a few drops of hydrochloric acid and was then dissolved in

water and diluted to 25 c.c. Of this solution 5 c.c., representing 2 c.c. of the original solution, were taken for a determination of the potassium, and 20 c.c., representing 8 c.c. of the original solution, for a determination of the calcium. For the potassium the delicate and satisfactory colorimetric method described in our previous paper was employed. For the calcium the solution was precipitated while warm with ammonia and ammonium oxalate. The precipitate of calcium oxalate after settling was washed repeatedly by centrifugalization, and was then dissolved in dilute sulphuric acid and titrated with a dilute standardized solution of potassium permanganate.⁴

In quite a number of our experiments the accelerator nerve failed to affect the heart beat, or at the best gave only one or two satisfactory accelerations and augmentations. In some of this group of experiments the perfusion was maintained for a number of hours and the liquids were analyzed as controls. In other cases stimulation of the accelerator caused very marked augmentation and acceleration of the beat, and the stimulation could be repeated successfully at intervals of several minutes for an hour or two. Why the nerve affected the heart in some cases and not in others was difficult to explain. In our successful experiments, all made during the spring and early summer, the following precautions were observed: (1) The accelerator nerve was kept warm during the intervals between stimulations by the application of warm cloths. (2) Care was taken in tying off the lungs not to lay the ligatures too close to the roots, since otherwise, especially on the left side, the accelerator nerve or some of its branches were caught in the ligature. Lastly, care was taken to use a solution which while somewhat strong in calcium (0.03 per cent) did not produce an independent beat of the ventricles. We had some reason for believing that in those hearts in which the ventricles beat independently of the auricles, the accelerator nerve failed to affect the heart. As this idea did not occur to us until our work was nearing completion, decisive experiments to test its correctness were not made in sufficient numbers to warrant a positive statement.

The results of our experiments seemed to prove conclusively that neither the calcium nor the potassium in the circulating liquid

⁴ The titrations for the calcium were all made for us by Mr. Burge, Fellow in Physiology, who was using the method in connection with another research. We desire to thank him for his kindness in making these determinations for us.

showed any variation in amount, after a perfusion lasting for hours, and after long-continued excitation of the heart through its accelerator nerve.

As an indication of the character of the results obtained, the protocols of two experiments may be given in detail, one of them being a control in which the accelerator was not stimulated, and one an experiment in which the accelerator was effective and was stimulated a great number of times.

Experiment, May 1, 1908. — Cat, etherized and bled. Heart isolated.

11 A. M., beat well upon the stock solution, but accelerator nerves on stimulation gave no effect. At 11.45 A. M. placed on a special supply of 150 c.c. which was maintained in continuous circulation. At 12.45 P. M. the heart was excised and suspended in a covered funnel so that all the drip could be secured. A little new solution was added to the supply flask and the circulation was continued until 4.45 P. M. The temperature of the circulating liquid varied from 35° to 37° C. At the end of the experiment the heart was still beating well. The circulating liquid was slightly turbid. On acidulation and boiling very little increase in opalescence could be detected. 10 c.c. were taken from the stock liquid and from the special supply of 150 c.c. which had been circulated through the heart for five hours. The solutions were evaporated to dryness, ashed, moistened with a few drops of HCl, again evaporated to dryness, dissolved in 25 c.c. water, of which 5 c.c. were used for determination of the potassium and 20 c.c. for the calcium.

Calcium. — 8 c.c. of original Locke's solution contained 0.955 mgm. Ca. 8 c.c. of the circulated solution contained 0.966 mgm. Ca.

Potassium. — In colorimeter the stock solution (B) compared with the standard solution of potassium chlorplatinite containing in each cubic centimetre 0.00265 mgm. K, gave the following reading:

$$\text{Standard} = 40; B = 49$$

Hence B contained 0.00216 mgm. K to each c.c. Since the solution had been diluted 50 times, the original solution contained $(0.00216 \times 50 \div 52.3)$ 0.21 mgm. KCl to each cubic centimetre, or 0.021 per cent KCl.

Compared with (B), the solution which had been perfused through the heart (A) gave the following reading:

$$A = 40; B = 39$$

Hence A contained 0.0105 per cent potassium compared with 0.0108 per cent in B, the liquid before circulation.

Experiment, May 21, 1908. — Young cat, etherized, bled. Heart isolated at 10.30 P. M., beat very well upon the stock solution. Accelerator nerve prepared on left side. Stimulus applied to the ganglion gave a very pronounced acceleration and augmentation of the heart beat. The heart was placed on the special supply (100 c.c.) at 11.15 A. M., and during the continuous circulation of this solution the accelerator nerve was stimulated successfully 38 times at intervals of from two to seven minutes, the stimulus being increased in strength at intervals, as follows:

First 18 stimulations, induction coil (Gaiffe's) at 50.
5 stimulations, induction coil (Gaiffe's) at 75.
10 stimulations, induction coil (Gaiffe's) at 100.
2 stimulations, induction coil (Gaiffe's) at 125.
3 stimulations, induction coil (Gaiffe's) at 150.

After more than two hours of the stimulation the heart was still beating very well, and was turned over to another observer for a different experiment. In spite of much cutting and handling it continued to beat well for five hours.

Ten c.c. were taken from the stock liquid and from the perfused liquid and treated as usual (see above) for the analyses of calcium and potassium. The calcium analyses were made in duplicate by Mr. Burge, and the potassium analyses in duplicate by Mr. Burge and by one of the authors (H.).

Calcium. —

8 c.c. of original stock solution contained I. 0.855 mgm. Ca.
II. 0.855 mgm. Ca.
8 c.c. of the circulated liquid contained I. 0.855 mgm. Ca.
II. 0.855 mgm. Ca.

Potassium. —

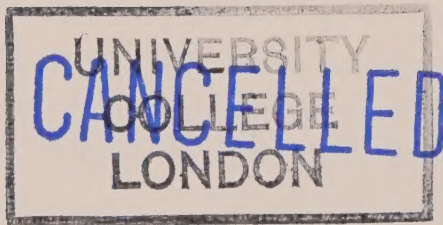
Analysis by H. Stock solution = 0.137 mgm. K per c.c.
Circulated solution = 0.132 mgm. K per c.c.
Analysis by B. Stock solution = 0.138 mgm. K per c.c.
Circulated solution = 0.136 mgm. K per c.c.

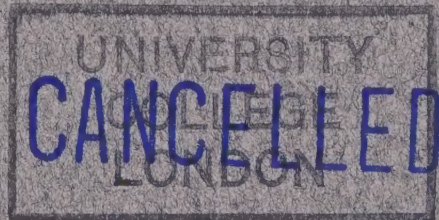
Nitrogen excretion in the isolated heart. — Burian⁵ has shown that in skeletal muscle there is apparently a continuous formation and elimination of hypoxanthin, the amount being increased when the muscle is made to contract. This result was obtained by perfusing the muscle with a circulating liquid composed of Ringer's solution and defibrinated blood. It seemed probable that in our

⁵ BURIAN: Zeitschrift für physiologische Chemie, 1904-1905, xliii, p. 532.

experiments in which the contracting heart was perfused for hours with a limited supply of Locke's solution decisive evidence might be obtained in regard to the production of purin bases or of other forms of nitrogenous waste. It was not possible at the time the experiments were made to examine separately the liquid that had been perfused through each heart, but the specimens of perfused liquid, after they had been acidified and boiled to remove protein, were brought together and kept, with the addition of a little toluene, until it was convenient to make an examination.

Dr. W. Jones was kind enough to examine this liquid for the purin bases, and he reports that no evidence could be obtained of a trace of such bodies. In this respect, therefore, the heart muscle seems to differ from the skeletal muscle, although possibly the difference may be due to the fact that in our experiments the hearts were supplied with a circulating liquid containing an abundance of dextrose. On the other hand, the perfused liquid, after being boiled with dilute sulphuric acid, gave very distinct evidence of the presence of creatinin when tested by Weyl's and Jaffe's reaction. The heart, therefore, must give off creatinin (or creatin) to the circulating liquid, and it will be interesting to determine how far this elimination is associated with functional activity.





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